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Patentanmeldung Nr. Patent application No. Demande de brevet n°

99117805.4

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A minimal tau peptide for the nucleation of paired helical filaments

The present invention relates to a method for identifying and obtaining an inhibitor, capable of modifying the PHF ("paired helical filaments") formation comprising the steps of (a) incubating a peptide comprising a specific tau derived peptide as defined herein or a fragment(s) thereof with a compound to be screened under conditions which permit assembly of said tau-derived peptides into nucleation sites for PHF assembly and/or into aggregation products; and (b) detecting the presence, decrease, or absence of nucleation sites for PHF assembly and/or the presence, decrease or absence of said aggregation products wherein said absence and/or decrease is indicative for putative inhibitors for PHF formation. Furthermore, the present invention provides inhibitors identified or obtained by said method as well as compositions comprising said inhibitor, wherein said composition is preferably a diagnostic and/or a pharmaceutical composition. The present invention further relates to a method for detecting and/or measuring PHF formation comprising the steps of (a) incubating a peptide comprising a specific tau derived peptide as defined herein or (a) fragment(s) thereof, with tau-proteins and/or fragments thereof under conditions which permit assembly of tau-proteins and/or fragments thereof into PHFs; and (b) detecting the presence, absence, decrease or increase of PHFs and/or nucleation sites of PHF assembly.

Several documents are cited throughout the text of this specification. Each of the documents cited herein (including any manufacturer's specifications, instructions, etc.) are hereby incorporated by reference; however, there is no admission that any document cited is indeed prior art of the present invention.

Alzheimer's disease and other dementias are characterized by abnormal protein deposits in the brain, such as amyloid plaques or neurofibrillary tangles, formed by fibrous assembli s of the Aβ peptide or of tau protein, wherein these tangles comprise tau protein aggregated into so-called paired helical filaments (PHFs) as reviewed in Lee and Trojanowski (1992), Curr. Opin. Neurobiol. 2, 653-656. These aggregates are thought to be toxic to neurons, either by causing some toxic signaling defect (in the case of Aβ) or by

(repeats plus flanking regions) constitutes the microtubule-binding domain. The core of the above-mentioned PHFs (paired helical filaments) are mainly built from the repeat domain (Ksiezak-Reding and Yen, N uron 6 (1991), 717-728; Novak, EMBO J. 12 (1993), 365-370; Wischik, Proc. Natl. Acad. Sci. USA (1988), 4506-4510), and this domain also promotes PHF assembly in vitro (Wille, J. Cell Biol. 118 (1992), 573-584). Tau contains almost no secondary structure but rather appears as a Gaussian random coil, as judged by spectroscopic and X-ray evidence (Cleveland, J. Mol. Biol. 116 (1977), 227-247). These data thus provide no basis for making assumptions about secondary structure interactions. Additionally, as e.g. shown in Schweers (1994), JBC 269, 24290-24297, tau protein behaves in solution as if it were denatured and no evidence for compact folding was detected. Schweers and colleagues employed a variety of spectroscopy methods in order to elucidate the conformation of tau protein and PHFs and came to the conclusion that it is unlikely that the aggregation of tau into Alzheimer PHFs is based on interactions between strands of β-sheets (a model currently favored for other disease-related polymers such as β-amyloid fibers of Alzheimer's disease). Therefore, the nature of the PHF (and/or tau) aggregation mechanism could not simply be modeled on the basis of known protein structures. On the other hand, despite its random coil appearance in solution, tau assembles into a well-defined periodic fiber, the paired helical filament (PHF). This process can be enhanced by oxidation (Wille (1992), loc. cit.), by polyanions (Friedhoff, Biochemistry 37 (1998a), 10223-10230; Goedert, Nature 383 (1996), 550-553; Kampers, FEBS Letters 399 (1996), 344-349; Perez, J. Neurochem. 67 (1996), 1183-1190) and can b described by a nucleation-condensation mechanism (Friedhoff, Proc. Natl. Acad. Sci. USA 95 (1998b), 15712-15717). Here, too, the secondary structure content is b low detectability (Schweers, J. Biol. Chem. 269 (1994), 24290-24297). A fraction of tau polymers in Alzheimer brains occurs as straight (untwisted) fibers (Crowther, Proc. Natl. Acad. Sci. USA (1991), 2288-2292), and fibers without axial periodicity have also been described for some in vitro assembly conditions (Montejo de Garcini and Avila, J. Biochem. 102 (1987), 1415-1421; Perez, loc. cit.; Wilson and Binder, Amer. J. Pathol. 150 (1997), 2181-2195).

Because of the long-range periodicity of most PHFs it can be speculated that they are built from a reproducible secondary structure lem nt in the microtubule-binding domain of tau which is responsibl for the repetitive and specific interaction that leads to filament aggregation. However, said secondary structure was not yet elucidated and this secondary

and oxidation of SH-groups on the tau protein or the corresponding fragments.

Considering the fact that tau aggregation is much less understood then that of, inter alia, β-amyloid, and even counter-intuitive since tau is cytosolic and one of the most highly soluble proteins known in the art, it is not clear why tau should aggregate in a specific manner to form Alzheimer related PHFs. Furthermore, it is unknown which specific structural principle could be responsible for PHF formation and/or aggregation. Additionally, prior test systems and methods, based on full-length tau or fragments thereof comprising the repeat domain are rather slow and are not capable of providing for fast and reliable assays for PHF formation and/or aggregation of tau or tau derived peptides. Said desired fast and reliable test assays may be used, inter alia, for the testing of potential drugs which may interfer with the above described pathological tau assembly. Accordingly, means and methods for diagnosing and treating Alzheimer's disease on the basis of said structural principle of tau, e.g. with chemotherapeutic treatment of said disease which influences tau aggregation, were hitherto not available but are nevertheless highly desirable.

Thus, the technical problem of the present invention is to comply with the needs described above. The solution to this technical problem is achieved by providing the embodiments characterized in the claims.

Accordingly, the invention relates to a method for identifying and obtaining an inhibitor, capable of modifying the PHF formation comprising the steps of

(a) incubating a peptide comprising a specific tau derived peptide as defined in any one of SEQ ID NOs 6, 7, 8 and 9 or (a) fragment(s) thereof, with a compound to be screened under conditions which permit assembly of said tau derived peptides into nucleation sites for PHF assembly and/or into aggregation products; and

(b) detecting the presence, decrease, or absence of nucleation sites for PHF assembly and/or the presence, decrease or absence of said aggregation products wherein said absence and/or decrease is indicative for putative inhibitors for PHF formation.

The term "PHF formation" as used in accordance with this invention means the assembly of tau protein or fragments thereof into paired helical filaments, wherein said paired helical filaments do not only comprise bona fide PHFs but also thin filaments of tau protein or fragments thereof which may serve as nuclei and/or nucleation sites which can fficiently promote the assembly of bona fide PHFs from tau fragments, tau constructs and/or intact

"PHF6/R2", derived from the human fetal tau sequence 275 to 280 (SEQ ID NO: 7). Another peptide comprising a specific tau derived peptide as defined herein above is, inter alia, shown in SEQ ID NO: 9 and denoted as "PHF8". As defined in the appended examples, said peptide comprises the amino acid sequence as depicted in SEQ ID NO: 8 and two additional amino acids (G and K) derived from the neighbouring amino acid sequence of SEQ ID NO: 8 in the full-length human tau sequence. Additionally, the term further comprises (a) peptide(s) or (a) (poly)peptide(s) which encompass amino acid chains of any length, wherein the amino acid residues are linked by covalent peptide bonds. These (poly)peptides, therefore, may consist entirely of the peptides as defined in the corresponding SEQ ID NOs 6, 7, 8 and 9 or may contain additional sequences. The additional sequences may (but need not) be sequences derived from tau protein, cytoskeletal proteins, microtuble associated proteins and/or microtuble binding proteins. Preferably, said amino acid chains comprises at least 6 amino acids, more preferably said amino acid chain comprises at least 8 amino acids, more preferably at least 10 amino acids, more preferably at least 15 amino acids, more preferably at least 20, more preferably at least 30, more preferably at least 40, even more preferably at least 45 and ven more preferably at least 50 amino acids. Most preferably, said amino acid chain comprises at least 60 amino acids. However, particularly preferred is an amino acid chain comprising 43 amino acids. In a particularly preferred embodiment said amino acid chain is the amino acid sequence as represented in SEQ ID NO: 6 comprising the amino acid sequence as represented in SEQ ID NO: 8 as well as amino acid sequences derived from tau. The term "or (a) fragment(s) thereof" in (a), supra, means in accordance with the present invention a peptide comprising a specific tau derived peptide fragment as defined in any one of the SEQ ID NOs 6, 7 or 8. Preferably, said fragment comprises at least 3 amino acids. Most preferably these amino acids are the amino acids V,Q,I (Valine, Glutamine, Isoleucine).

Within the scope of the present invention are also peptides comprising tau derived peptides which are homologous to the above described peptides as depicted in SEQ ID NOs: 6 to 9, but are derived from different species than human. Especially preferred tau homologues are in this context tau-derived peptides from mouse, rat, cow, sheep, polar bear, etc. Furthermore, the present invention also relates to methods, compositions, us s and kits wherein said peptide comprising (a) tau derived peptide(s) is a peptide which is similar to the above described peptides, but wherein said amino acids V, Q and/or I (Valine, Glutamin , Isoleucine) have been replaced, either alone or in combination, by

defined in any one of SEQ ID NOs" can comprise (a) further domain(s), said domain(s) being linked by coval nt or non-covalent bonds. The linkage can be based on genetic fusion according to the m thods known in the art (Sambrook et al., loc. cit.; Ausubel, loc. cit.) or can be performed by, e.g., chemical cross-linking as described in, e.g. WO 94/04686. The additional domain present in the fusion protein/peptide comprising the above mentioned peptide comprising a specific tau derived peptide may be joined directly (i.e. with no intervening amino acids) or may be linked by a flexible linker, advantageously a polypeptide linker, wherein said polypeptide linker comprises plural, hydrophilic, peptide-bonded amino acids of a length sufficient to span the distance between the C-terminal end of said further domain and the N-terminal end of the peptide or vice versa. Said polypeptide linker might be a cleavable linker, e.g. a linker cleavable by specific enzymes. Said fusion protein/peptide may comprise other functional amino acid sequences, like protein tags (GST, GFP, h-myc-peptide, FLAG, etc.) which are derived from heterologous proteins. These further protein tags may be used, inter alia, in adopted detection systems as described herein in the appended examples.

The term "nucleation sites for PHF assembly" as used in accordance with the present invention means the complex or aggregate formation between at least two peptides comprising a specific tau derived peptide as defined in any one of SEQ IDs 6, 7, 8, 9 or 57 or (a) fragment(s) thereof. The term furthermore comprises short or thin filaments formed in vitro without a paired helical appearance but a high competence to nucleate bona fide PHFs from full length tau and/or fragments thereof. Therefore, the term "nucleation sites for PHF assembly" comprises short dimers (homodimers or heterodimers) of peptides comprising said tau derived peptides or fragments thereof as well as "nuclei" of high r structure which can then elongate into PHFs. The detection of the presence, decrease or absence of such nucleation sites for PHF assembly is well known in the art as described in Friedhoff (1998a, b) loc. cit. or is illustrated in the appended examples. The detection can be carried out, inter alia, by microscopic and/or spectroscopic methods as explained herein below. Further particular preferred detection methods comprise fluorescenc spectroscopy, especially fluorescence spectroscopy employing thioflavine S or T or the detection of the self-fluorescence of tyrosine (like Tyr310 of the human tau sequence, see, inter alia PHF6 or PHF8 as depicted in SEQ ID NOS: 7 and 8, or K19 fragments as depicted in SEQ ID NO: 6 or 57), as described in the appended examples. Said detection of tyrosine self-fluorescence is particularly preferred when short tau-derived peptides and

In addition, oligonucleotides and/or aptamers which may specifically bind to the above mentioned peptide(s) and thereby modify and/or inhibit the PHF-formation in vitro and/or in vivo are also envisaged as potential inhibitors to b tested in the method of the present invention. The term "oligonucleotide" as used in accordance with the present invention comprises coding and non-coding sequences, it comprises DNA and RNA and/or comprises also any feasible derivative. The term "oligonucleotide" further comprises peptide nucleic acids (PNAs) containing DNA analogs with amide backbone linkages (Nielson, Science 274 (1991), 1497-1500). Oligonucleotides which may inhibit PHF-formation and which can be identified and/or obtained by the method of the present invention can be, inter alia, easily chemically synthesized using synthesizers which are well known in the art and are commercially available like, e.g., the ABI 394 DNA-RNA Synthesizers.

In accordance with the present invention, the term aptamer means nucleic acid molecules that can bind to target molecules. Aptamers commonly comprise RNA, single stranded DNA, modified RNA or modified DNA molecules. The preparation of aptamers is well known in the art and may involve, inter alia, the use of combinatorial RNA libraries to identify binding sites (Gold, Ann. Rev. Biochem. 64 (1995), 763-797).

Potential other inhibitors to be screened with the method of the present invention include small molecules which bind to, interfere with and/or occupy relevant sites on tau, fragments thereof and/or on peptides which are derived from tau. Furthermore, said inhibitors comprise molecules capable of interfering with amino acid sequences which are derived from the tau protein sequence and comprise the amino acid sequences as depicted in SEQ ID NOs: 6 to 9 or 57. In particular said inhibitors interfere with the potential β-sheet formation of peptides as depicted in SEQ ID NOs: 7, 8 or 9. Such inhibitors comprise also molecules which bind to other parts of the full-length tau sequence but inhibit the correct folding of said tau protein (and/or fragments thereof) and ther by inhibit the formation of correct β-structures on and/or within the amino acid sequences as depicted in SEQ ID NOs: 7, 8 or 9. Said molecules might be selected prior to employing the method of the present invention, inter alia, by well known ELISA tests and/or screening methods like spot membrane binding assays, as exemplified, inter alia, in the appended examples. Examples of small molecules include, but are not limited to small p ptides or peptide-like molecules.

antibodies, antibody fragments, antibody derivatives, aptamers, specific oligonucleotid s and/or small mol cules which specifically bind to/interact with said peptide. Said small molecules may comprise, inter alia, synthetically produced peptide-analogs which may comprise, inter alia D-amino acids.
Said "measuring" in step (c) may be carried out by specific immunological and/or biochemical assays which are well known in the art and which comprise, e.g., homogenous and heterogenous assays as described herein below. A further method which may be employed comprises FRET (fluorescence resonance energy transfer; as described, inter alia, in Ng, Science 283 (1999), 2085-2089).

In a preferred embodiment, the present invention relates to the above described method wherein said measuring step (c) comprises the measurement of a formation of a second complex of said tau-derived peptide and said compound.

The measurement of a complex formation is well known in the art and comprises, inter alia, heterogeneous and homogeneous assays. Homogeneous assays comprise assays wherein the binding partners remain in solution and comprise assays, like agglutination assays (see herein below). Heterogeneous assays comprise assays like, inter alia, immuno assays, for example, ELISAs, RIAs, IRMAs, FIAs, CLIAs or ECLs.

Further methods for measurement comprise the monitoring of complex and/or aggregate formation in real time by the fluorescence of dyes such as thioflavine S or T (as described in Friedhoff (1998a, b) loc. cit., or as illustrated in the appended examples), or the measurement of self-fluorescence, like the self-fluorescence of tyrosine (as illustrated in the appended examples).

In a more preferred embodiment, the method of the invention is a method wherein said measuring step (c) comprises the measurement of the amount of said first molecule that is not bound to said tau-derived peptide.

Again said measurement may comprise a plurality of measuring and/or detection methods which are well known in the art. For example, said first molecul and therefore said amount may be measured by immuno detection methods or by the detection of a label which was added to said first molecule. Said label may be, inter alia, a tag, a fluorescent

Thus, in addition, the present invention relates to a method for the preparation of a pharmaceutical composition comprising the steps of a method described herein above and synthesizing and/or formulating the compounds identified, obtained and/or screened in step (b) or derivative thereof in a pharmaceutically acceptable form. The therapeutically useful inhibitor/compound identified according to the method of the invention may be formulated and administered to a patient as discussed herein below. For uses and therapeutic doses determined to be appropriate by one skilled in the art, see infra.

In a more preferred embodiment, the method of the invention relates to a method wherein said peptide comprising a tau-derived peptide is bound to a solid phase.

The method of the invention is preferably carried out in the liquid phase, however, said peptide can be bound to a solid phase/a solid carrier. Additionally, as pointed out herein above, said peptide can be additionally labeled in various ways, inter alia, by fluorescence or radio labels. Said solid phase comprises, but is not limited to, a plastic, a glass, a silicon, a colloidal metal, a cellulose and/or a polymeric support. Said solid support is s lected from the group consisting of solid organic polymers, cellulose/cellulose-based membranes, colloidal metal particles, plastic surfaces, or any combination thereof. A number of supports known in the art are suitable for serving the purposes of the pres nt invention. Such supports may comprise, inter alia, membranes, plates, stripes, wells, beads, microchips or containers. Suitable materials for such supports or materials for further coating of said supports include, but are not limited to, glass, polystyrene, polyvinyl chloride, polypropylene, polyethylene, polycarbonate, latex, dextran, nylon, amyloses, natural and modified celluloses, like nitrocellulose, polyacrylamide, agaroses, magnetide and metals. The above mentioned colloidal metal particle may be, inter alia, a gold particle, and said above mentioned plastic surface comprises the well of a microtiter plate. Additionally, said above mentioned solid organic polymer may be or comprise a latex bead. Combinations of several supports, such as, e.g. latex beads and colloid metal particles, are also within the scope of the present invention.
Said particles bound to solid supports/phases are useful in the method of the present invention when, inter alia, said detection and/or measuring step is carried out by homogen ous assays, for example by an agglutination assay which measures the formation of a complex of said peptides. An example of such a homogeneous assay would be a latex enhanced turbimetric assay. In accordance with the present invention it might

herein above and, optionally, further molecules, either alone or in combination, like e.g. molecules which are capable of interfering with the formation of neurofibrillary tangles and/or the formation of amyloid plaques. The composition may be in solid, liquid or gaseous form and may be, inter alia, in a form of (a) powder(s), (a) tablet(s), (a) solution(s) or (an) aerosol(s). In a preferred embodiment, said composition comprises at least two, preferably three, more preferably four, most preferably five inhibitors as defined herein above.

In a preferred embodiment, said composition is a diagnostic or a pharmaceutical composition.

The pharmaceutical composition may further comprise a pharmaceutically acceptable carrier, excipient and/or diluent.

Examples of suitable pharmaceutical carriers are well known in the art and includ phosphate buffered saline solutions, water, emulsions, such as oil/water emulsions, various types of wetting agents, sterile solutions etc. Compositions comprising such carriers can be formulated by well known conventional methods. These pharmaceutical compositions can be administered to the subject at a suitable dose. Administration of the suitable compositions may be effected by different ways, e.g. by intravenous, intraperitoneal, subcutaneous, intramuscular, topical, intradermal, intranasal or intrabronchial administration. Most preferably, said administration is intra-cerebral and/or by an administrative route which by-passes the blood/brain barrier. The dosage regimen will be determined by the attending physician and clinical factors. As is well known in the medical arts, dosages for any one patient depends upon many factors, including the patient's size, body surface area, general health, age, sex, the particular compound to be administered, time and route of administration, and other drugs being administered concurrently. Pharmaceutically active matter may be present, inter alia, in amounts between 1 ng and 100 mg per dose; however, doses below or above this exemplary range are envisioned, especially considering the aforementioned factors.

The compositions of the invention may be administered locally or systemically. Administration will generally be parenterally, e.g., intravenously. The compositions of the invention may also be administered directly to the target site, e.g., by biolistic delivery to an internal or external target site (like the brain) or by catheter to a site in a (brain) art ry. Furthermore, the pharmaceutical composition of the invention may comprise further

co-factors which might promote PHF assembly/formation and/or formation of nucleation sites for PHF assembly may be added. Such co-factors are well known in the art and comprise, e.g., anionic co-factors, like polyglutamate, RNA or heparin (see, inter alia, Friedhoff (1998a), loc. cit.). The detection/measuring step (b) may be carried out as described herein and in the appended examples and may comprise microscopic and/or spectroscopic methods.

In a more preferred embodiment, the detection and/or measuring step in the method of the present invention is carried out by microscopic or spectroscopic means. In a yet more preferred embodiment said microscopic means comprises electron microscopy and said spectroscopic means comprises circular dichroism spectroscopy or fluorescence spectroscopy. In a particular preferred embodiment said fluorescence spectroscopy comprises a thioflavine S or T assay or a tyrosine self-fluorescence-assay, as described herein. Said microscopic and/or spectroscopic means are well known in the art as illustrated, inter alia, in the appended examples. Further detection methods are described in the art and comprise methods as described in the appended examples. As mentioned herein above, suitable methods comprise not only microscopic and/or spectroscopic means but comprise also heterogeneous or homogeneous assays, like ELISAs or agglutination assays.

In yet another embodiment, the present invention relates to a kit comprising at least one of the following:

(a) a peptide comprising a specific tau derived peptide as defined in any one of SEQ ID NOs 6, 7, 8 and 9 or (a) fragment(s) thereof;

(b) tau-protein(s) and/or fragments thereof as defined herein above;

(c) a solid support as defined herein above,

adopted for carrying out the method of the invention, optionally further comprising a standard and/or suitable means for detection.

Furthermore, the present invention relates, in another embodiment, to a kit comprising an inhibitor or a composition as defined herein above. Such (a) kit(s) of the present invention may comprise a carrier means being compartmentalized to receive in close confinement one or more container means such as vials, tubes and the like, each of the container means comprising one of the separate elements to be used in the method of the invention

Nos: 6 to 9. Such compounds are good candidate inhibitors of PHF formation of the present invention. Other screening and/or identification methods are well known in the art and comprise, inter alia, phage display (as described, inter alia in US 5,541,109) or affinity-labelings via cross-linkers. Furthermore, the method of the present invention is therefore also useful in and can easily be adapted to high-throughput screenings and/or computerized screening assays.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integer or step.

The figures show:

Fig. 1 Bar diagram of tau constructs and peptides. (a) htau40, the longest isoform in the human CNS, containing four repeats of ~31 residues (labeled 1-4) and two inserts near th N-terminus. Residue numbering follows that of htau40 throughout. (b) htau23, the smallest and fetal isoform, lacking the N-terminal inserts and repeat R2. (c) K19 represents only the repeat domain of htau23, i.e. R1, R3, R4 (i.e. 94 residues, Q244-N368, but without V275-S305). (d) PHF43, a subfragment of K19, containing the end of R1, all of R3, and the beginning of R4, but without R2 (43 residues). (e) PHF8, 8 residues spanning the junction between R1 and R3. (f) PHF6, 6 residues from the beginning of R3. The oth r peptides (V318-K321, V318-G335 and G335-E342) span the PHF43 sequence and were used for analysis by CD.

Fig. 2: Kinetics of PHF assembly. The assembly of tau or tau fragments was measured fluorimetrically, using the fluorescence of ThS (Friedhoff et al., 1998a). Protein concentrations were 20µM, in the presence of 5µM heparin and 20 mM NH_4Ac, at room temperature. PHF43 assembles rapidly and spontaneously (diamonds, top curve, $t_{1/2}$ = 0.75 min), but htau23 is very slow by comparison (circles, bottom curve, $t_{1/2}$ = 180 min). K19 shows intermediate kinetics (triangl s, $t_{1/2}$ = 12 min). Htau23 can b speeded up to a

Fig. 5: Peptide spot membran interactions between PHF43 and peptides d rived from the repeat region of tau.

The region of tau from L253 in repeat 1 to D348 in repeat 4 (encompassing the sequence of PHF43) was subdivided into consecutive 15-mer peptides staggered by 3 residu s, synthesized and covalently attached to a cellulose membrane (Frank, J. Biotechnol. 41 (1995), 259-272), and incubated with iodinated PHF43. The bars represent the bound radioactivity determined by autoradiography. The strongest interaction occurs around spot 4-25 which contains the sequence of PHF6 (VQIVYK, bold) at the beginning of R3. Another cluster of strong interaction occurs around spot 4-16 which harbors the analogous sequence in R2 (VQIINK, bold).

Fig. 6: CD spectroscopy of tau constructs and tau polymers. CD spectra w re obtained at 50 µM protein concentrations in 10 mM NH_4-acetate at room temperature, in the absence or presence of 50 µM heparin and/or 50% TFE.

(a) htau23 monomers (circles), dimers (triangles), and assembled PHFs (solid line) show similar CD curves with or without heparin (filled or open symbols). The minimum is around 200 nm, indicating that a random coil structure predominates independently of dimerization or polymerization.

(b) K19 monomers (SEQ ID NO: 10; without heparin open circles, with heparin filled circles) and dimers (without heparin open triangles, with heparin filled triangles) show similar CD curves, with a mostly random coil structure (minimum around 200 nm), except after polymerization with heparin (solid line) which indicates an increase in beta she t structure.

(c) PHF43 monomers (SEQ ID NO: 6; without heparin open circles, with heparin fill d circles) and dimers without heparin (open triangles) show mostly random coil structure, but a noticeable shift to beta sheet structure when dimers are assembled into PHFs in the presence of heparin (solid line).

(d) PHF8 (SEQ ID NO: 9; without heparin open circles, with heparin filled circles) and PHF6 (SEQ ID NO: 8; without heparin open triangles, with heparin filled triangles) show mostly random coil structure, but shift to more beta sheet structure (especially PHF6) upon polymerization in the presence of heparin (compare Fig. 2).

(e) Three peptides (V_{313}DLSKVTSK$_{321}$, SEQ ID NO: 17; without heparin open circles, with heparin filled circles, V_{318}TSKCGSLGNIHHKPGGG$_{335}$ SEQ ID NO: 18 without heparin open triangles, with heparin filled triangles, G_{335}QVEVKSE$_{342}$, SEQ ID NO: 19; without

EXAMPLE I

Proteolytic degradation products of the repe t domain caus fficient assembly of PHFs

Earlier efforts to reconstitute PHFs in vitro had shown that tau constructs containing roughly the repeat domain polymerized much more readily than the full-length protein (Wille, loc. cit.), consistent with the observation that the repeat domain forms the core of PHFs from Alzheimer brains (Wischik, loc. cit.). To achieve PHF assembly from intact tau it was necessary to add polyanionic cofactors such as heparin, RNA, or poly-Glu (Goed rt, loc. cit.; Kampers, loc. cit.; Perez, loc. cit.). However, it was noted that different preparations of tau protein showed a considerable variability in their assembly efficiency, and that some of the more readily aggregating preparations contained proteolytic breakdown products, as judged by SDS gels. Tau constructs were digested with different proteases, the breakdown products were characterized and they were analyzed for their assembly behaviour. In this study, assembly behaviours were studied as follows: varying concentrations of tau isoforms or tau constructs and fragments (typically in the range of 1-100 µM) in volumes of 20-500 µl were incubated at 37°C in 50 mM NH_4Ac, pH 7.0 or 20 mM MOPS-NaOH, pH 7.0 containing anionic cofactors (polyglutamate, RNA or heparin) as described (Friedhoff, loc. cit.). Incubation times varied between minutes up to sev ral days. PHF formation was always confirmed by electron microscopy. Here, construct K19, a derivative of the fetal tau isoform (htau23) was employed for assembly studies. K19 contains only three repeats (R1, R3, R4; the second repeat R2 is absent due to alternative splicing; Fig. 1). Construct K19 (SEQ ID NO: 10) was expressed, like human tau 23, in E.coli as described (Biernat et al, EMBO J. 11 (1992), 1593-1597). The proteins were expressed and purified as described in the art, employing heat stability and FPLC Mono S (Pharmacia) chromatography, as described, inter alia, in Gustke (1994), Biochemistry 33, 9511-9522. The purity of proteins was verified by SDS-PAGE. Protein concentrations were determined by Bradford assay. Synthetic peptides were obtained from Eurogentec SA (Seraing, Belgium). Construct K19 was digested with chymotrypsin, trypsin, AspN, and GluC. Briefly, different fragments of K19 were generated by proteolysis with the above mentioned proteases. Extensive proteolysis by trypsin (ratio w/w 1:100, in 50 mM NH_4CO_3 pH 8.4, 37°C for 2 h), limited proteolysis by chymotrypsin (ratio w/w 1:200, in 50 mM NH_4CO_3 pH 8.4, RT, for 30 min) and extensive proteolysis by GluC (w/w 1:100, in 50 mM

heparin, RNA, or poly-Glu), as well as initial dimerization by oxidation of Cys322 into disulfide bridges, indicating that dimers form the effective building blocks of assembly (Fri dhoff, loc. cit.; Schweers, loc. cit.). In fact PHF43 showed an unusually strong tendency to dimerize (in the absence of DTT) so that more than half was already dimerized upon digestion of K19, and after elution from the reverse phase column the dimer fraction was close to 100%. The reaction was based on the crosslinking of Cys322, th only sulfhydryl group in K19 or PHF43, and could be reversed by reducing agents (e.g. DTT).

EXAMPLE II

PHF43 alone forms straight filaments but rapidly nucleates bona fide PHFs from tau23

Electron microscopy was carried out as described herein above. It could be shown that most fibers obtained after self-assembly of the peptide PHF43 (see Example I) appeared as straight thin filaments, often aggregated laterally, and lacking the periodic ~80 nm supertwist that is characteristic of Alzheimer PHFs, which is in contrast to fibers assembled from construct K19 or tau23. Since PHF assembly follows a nucleation-condensation mechanism (Friedhoff, Proc. Natl. Acad. Sci. USA (1998b), 15712-15717), fragments of PHF43 fibers were prepared by sonication (sonicator: Branson scientific; 2 min; output level 5; duty cycle 50%; as described in Friedhoff (1998b), loc.cit.) and it was asked whether these could function as seeds for PHF assembly from larger tau constructs or full-length htau23. Surprisingly, the seeds made from the fragmented PHF43 filaments were capable of rapidly nucleating bona fide PHFs from K19 or htau23, displaying the typical 80 nm twist (Fig. 2, 4). This means that the interaction between PHF43 molecul s must be very similar to that of PHFs, both in a kinetic sense (requiring dimerization and polyanions) and in a structural sense (nucleation of twisted fibers). The smaller diameter of PHF43-fibers could be accounted for by the small size of the peptide, compared to the larger tau constructs and isoforms studied previously (Kampers, loc. cit.; Wille, loc. cit.).

Therefore, PHF43, representing the third repeat plus short flanking sequences of tau ass mbles within seconds into thin fibers under the same conditions as full-length tau (favored by dimerization and anionic cofactors such as heparin, Fig. 2). Seeds d rived by

region around residue 310 tends to dimerize. A motif overlapping with the interacting peptides was the hexapeptide ^{306}VQIVYK^{311} (termed PHF6, bold in Fig. 5, SEQ ID NO: 8), at the beginning of R3. A second maximum of interaction was centered around Ile278, covering the sequence ^{275}VQIINK^{280} (SEQ ID NO: 7). This sequence is the equivalent of PHF6 at the beginning of R2 (present only in 4-repeat tau and included in the spot membrane). Remarkably, PHF6 in R3 and its counterpart in R2 represent the only regions in tau where a beta conformation is strongly predicted, consistent with the local clustering of residues with high β-sheet propensity such as V, I, Q, and Y. The PHF-forming interaction between tau or tau-derived peptides is strongly dominated by the hexapeptid ^{306}VQIVYK^{311} (PHF6), derived from the third repeat R3, or the equivalent sequence ^{275}VQIINK^{280} (now termed PHF6*) from the second repeat R2 (Fig. 5).

EXAMPLE IV

CD spectroscopy of tau fragments suggests a β-structure in PHF assembly conditions

The conformation of proteins can be probed by circular dichroism, infrared spectroscopy, or solution X-ray scattering. Earlier studies (Schweers (1995), loc.cit.; Wille (1992), loc.cit.) of tau isoforms had shown that the ordered secondary structure of tau (α-helix or β-sheet) was minimal, below the reliable detection limit of the methods (~5-10%). This had given rise to the notion that tau was a largely random structure, without a well-defined folding pattern, reminiscent of a Gaussian polymer (Schweers, loc. cit.). This view did however not exclude the possibility of a locally ordered structure which should become more visible with shorter tau-derived peptides. Therefore, the CD spectra of tau and its fragments in several conditions (Fig. 6 a-f and Fig. 7) were studied. All measurements were made with a Jasco J-710 CD-Dichrograph (Jasco, Japan) in a cuvette with 0.05 cm path length. For each experiment, 10 spectra were summed up and the molar ellipticity was determined after normalizing for the protein concentrations. The secondary structure interpretation of the CD-data was performed according to Chou and Fasman (Annu. Rev. Biochem. 47 (1978), 251-276), as implemented in the program Dicroprot (D leage and Geourjon, Comput Appl Biosci, 9 (1993), 197-9).

The above mentioned conditions for CD spectra experiments of tau and its fragm nts

dominates in all cases, even after PHF assembly (solid curve in Fig. 6a).Even if a local beta-structure were formed during PHF assembly, this does not become noticeable with full-length tau. The situation changes as the tau constructs become smaller (Fig. 6b): K19 also displays a mostly random coil structure in the monomeric or dimeric state, even with heparin, but polymerization induces a noticeable change in the spectra so that the minimum becomes wider and is shifted towards higher wavelengths, indicating a substantial change from random coil to beta structure. This behavior is reiterated in the case of PHF43 (Fig. 6c), showing mostly random coil structures, except after PHF assembly where the content of beta-structure is increased. Fig. 6d compares the curves of PHF6 (VQIVYK) before and after assembly through addition of heparin, as well as PHF8 (= PHF6 plus the preceding two residues, GKVQIVYK, SEQ ID NO: 9); again there is a substantial change from random coil to beta structure after assembly. Note that in th latter two cases the intermolecular interaction is not preceded by disulfide oxidation since Cys322 is not present. Next several peptides derived from the repeat domain of tau were probed. The nonapeptide ^{313}VDLSKVTSK^{321} (SEQ ID NO: 14) from R3 following aft r PHF6 does not polymerize and shows mostly random coil with some beta-structure in th presence or absence of heparin. The same is true for the 18-mer peptide ^{318}VTSKCGSLGNIHHKPGGG^{335} (SEQ ID NO: 15) which represents the second half of R3 that is more conserved between the repeats (sometimes termed the "repeat" in the strict sense, peptide provided by R. Hoffmann and L. Otvos, see Hoffmann, J. Pept. Res. 50 (1997), 132-142). Note that this peptide does not assemble even though it contains Cys322, indicating that the vicinity of Cys alone is not sufficient for PHF formation. Finally, the peptide ^{335}GQVEVKSE^{342} (SEQ ID NO: 16) shows a similar behavior, i.e. mostly random coil with some beta-structure, with or without heparin, and no assembly. This peptide is located at the beginning of R4, analogously to PHF8, but evidently does not have the capacity to interact with itself, presumably because it contains sheet-breaking residues. (Note that for the non-assembling peptides the assignment of beta-structure cannot be taken literally since no beta-sheets are formed. The numbers are merely generated by the computer algorithm whose theoretical assumptions are only partly fulfilled here).

In a further set of experiments the influence of the helix inducer TFE (50%) on the CD spectra was tested. The rationale was that monomeric or dimeric molecules should show a substantial conversion to helical structur , whereas polymerized fibers should be less

present in all tau isoforms and provides an explanation why all tau isoforms can form PHFs. The counterpart PHF6* lies at the beginning of R2 and is therefore present only in 4-repeat isoforms. If the two hotspots for β-sheet formation operated in a cooperative fashion they could possibly enhance PHF assembly, and this might explain why PHF formation is enhanced in dementias where the 4-repeat isoforms are overrepresented (e.g. FTDP-17, see Clark *et al.*, 1998; Hutton *et al.*, 1998; Spillantini *et al.*, 1998). For exampl , PHF6 and PHF6* could form an intra-molecular sheet-like interaction, ready to be propagated to neighboring tau molecules. This would imply a local hairpin fold, with a bend possibly at the flexible PGGG motif at the end of R2. The occurrence of such a fold has been suggested by previous fluorescence transfer experiments involving the cysteines in repeats R2 and R3 (Schweers et al., 1995).

(2) Dimerization of tau via oxidation of Cys322 into an inter-molecular disulfide bridge strongly promotes PHF assembly (Wille *et al.*, 1992). In 4R tau there are two cysteines (Cys291 in R2 and Cys322 in R3) which can either form inter-molecular bridges (Cys322-Cys322) which favors PHF assembly, or intra-molecular bridges (Cys291-C322) which locks tau in a folded conformation that is unfavorable for PHF assembly (Schweers *et al.*, 1995). Independently of that, the results shown here argue that the peptide containing Cys322 (peptide 318-335, Tab. 2) does not promote PHF assembly by itself. However, dimerization at Cys322 could bring two PHF6-motifs into close vicinity which would facilitate beta-sheet interactions. In this view, dimerization would act as an effective enhancer of local tau concentration. The close neighborhood of Cys322 and the PHF6 motif suggests that the molecules would form a parallel beta-sheet.

(3) The role of heparin or other anionic cofactors remains unknown in detail, but could effectively help reduce the repulsion between the cationic tau protein in a general way, as discussed by several authors previously (Friedhoff loc. cit., 1998a; Friedhoff loc. cit., 1998b; Goedert loc. cit., 1996; Hasegawa, J. Biol. Chem. 272 (1997), 33118-33124; Kampers, loc. cit.; Perez, loc. cit.). This would also be equivalent to enhancing the effective local concentration of tau. Heparin does not appear to change the conformation of tau by itself, as judged by the CD spectra, because a shift from random-coil to beta-structure is only observed as polymerization progresses.

(4) In a previous study (Schweers, loc. cit.) it was described that tau is a natively unfolded

EXAMPLE V

Aggregation assay of tau and tau-d rived peptides and detection by tyrosin s lf-fluorescence (Tyrosin-Assay)

This assay is applicable particularly to the aggregation of small peptides (e.g. PHF6 as shown in SEQ ID NO: 8; K19-fragments as depicted, inter alia, in SEQ ID NO:57 or 6; K19 as shown in SEQ ID NO:10; PHF43 as depicted in SEQ ID NO:6; PHF8 as shown in SEQ ID NO:9). It can be performed in a cuvette or a 384 well plate. The assay is aplicable to all tau isoforms and fragments containing Tyr310, but is particularly suitable for constructs d rived from the repeat domain which contain only this one tyrosine 310, e.g. K19 (Fig. 8a), PHF43 (Fig. 8b), PHF8 (Fig. 8c) or PHF6 (Fig. 8d). This assay is based on the fact that the environment of Tyr310 changes during aggregation. As a result of the aggregation the excitation maximum of the intrinsic tyrosine fluorescence (observed at the emission maximum at 310 nm) shows a red-shift by about 5nm (e.g. from 278 to 283 nm).

Reagents for 50 µl reaction volume:
17.5 µl H_2O
5 µl buffer NH_4Ac 200 mM, pH 7.0 (final concentration 20 mM)
25 µl heparin MW 3000, 1 mM (final concentration 500 µM)
2.5 µl peptide (e.g. K19, PHF43, PH19, PH8, or PHF6), 10 mM (final concentration 500 µM)

The reaction volume is placed in a fluorimeter (e.g. Fluoromax, ISA GmbH). The increase in aggregation can be measured by observing the excitation spectrum between 250 and 300 nm (Fig. 8a-d; figures show the excitation spectra before and after aggregation) at a fixed emission wavelength (310 nm).

EXAMPLE VI

Aggregation analysis of full-length-tau (isoform htau 23), fragments thereof comprising the repeat domain, PHF43 and PHF6

Human full-length tau23 is expressed in E. coli, purified as described (e.g. Friedhoff,

volume):
34 µl H_2O
5 µl buffer NH_4-acetate, 200 mM, pH 7.0;
1 µl Thioflavine S, 100 µM (final concentration 2 µM),
10 µl K19 protein from aggregation reaction (see above), 50 µM (final concentration 5 µM).

The solution is incubated for 10 min, placed in a cuvette in a fluorimeter (Fluoroskan Ascent, Labsystems) at room temperature. The fluorescence of Thioflavine S bound to the aggregated protein is determined at an excitation wavelength of 440 nm and an emission wavelength of 510 nm (see examples herein above). The degree of aggregation is determined by reference with a previously determined calibration series. As an additional control the presence of aggregated protein is observed by negative stain electron microscopy.

Human tau fragment PHF43 (SEQ ID NO: 6) is expressed in E. coli, purified as described above, and stored in 10 mM NH_4-acetate buffer pH 7.0. The aggregation experiment is set up as follows (for 50 µl reaction volume):
35 µl H_2O
5 µl buffer NH_4-acetate 200 mM, pH 7.0 (final concentration 20 mM)
5 µl heparin MW 3000, 500 µM (final concentration 50 µM)
5 µl PHF43 protein 500 µM (final concentration 50 µM)
The solution is incubated at room temperature for 4 min, during which time the protein aggregates.

The detection of aggregation by fluorescence is set up as follows (for 50 µl reaction volume):
34 µl H_2O
5 µl buffer NH_4-acetate, 200 mM, pH 7.0;
1 µl Thioflavine S, 100 µM (final concentration 2 µM),
10 µl PHF43 from aggregation reaction (see above), 50 µM (final concentration 5 µM).

The solution is incubated for 10 min, placed in a cuvette in a fluorimeter (Fluoroskan Ascent, Labsystems) at room temperature. The fluorescence of Thioflavine S bound to the aggregated protein is determined at an xcitation wavelength of 440 nm and an emission

Claims

1. A method for identifying and obtaining an inhibitor, capable of modifying th PHF formation comprising the steps of
   (a) incubating a peptide comprising a specific tau derived peptide as defined in any one of SEQ ID NOs 6, 7, 8 and 9 or (a) fragment(s) thereof, with a compound to be screened under conditions which permit assembly of said tau-derived peptides into nucleation sites for PHF assembly and/or into aggregation products; and
   (b) detecting the presence, decrease, or absence of nucleation sites for PHF assembly and/or the presence, decrease or absence of aggregation products wherein said absence and/or decrease is indicative for putative inhibitors for PHF formation.

2. The method of claim 1 wherein in step (a) said incubation is carried out in the presence of assembly-competent tau protein(s) and/or assembly-competent tau protein fragments and wherein in step (b) the presence, decrease and/or absence of PHF or tau filaments is detected.

3. A method for identifying and obtaining an inhibitor of PHF formation comprising the steps of:
   (a) contacting a peptide comprising a specific tau-derived peptide as defined in any one of SEQ ID NOs. 6, 7, 8 or 9 or (a) fragment(s) thereof with the first molecule known to bind to said peptide comprising a specific tau-deriv d peptide to form a first complex of said peptide and said first molecule;
   (b) contacting said first complex with a compound to be screened; and
   (c) measuring whether said compound displaces said first molecule from said first complex.

4. The method of claim 3 wherein said measuring step (c) comprises the measurement of a formation of a second complex of said tau-derived peptide and said compound.

any one of SEQ ID NOs. 6, 7, 8 or 9 or (a) fragment(s) thereof, with tau-protein(s) and/or fragments thereof under conditions which permit assembly of tau-proteins and/or fragments thereof into PHFs; and

(b) detecting the presence, absence, decrease or increase of PHFs and/or nucleation sites of PHF assembly.

16. The method of any one of claims 1 to 8 or 15, wherein said detection or measuring step is carried out by microscopical or spectroscopical means.

17. The method of claim 16 wherein said microscopical means comprises electron microscopy.

18. The method of claim 16 wherein said spectroscopical means comprises circular dichroism spectroscopy or fluorescence spectroscopy.

19. The method of claim 18, wherein said fluorescence spectroscopy comprises a thioflavin S or T assay or a tyrosine self-fluorescence assay.

20. A kit comprising at least one of the following:

(a) a peptide comprising are specific tau derived peptide as defined in any one of SEQ ID NOs 6, 7, 8 or 9 or (a) fragment(s) thereof;

(b) tau-protein(s) and/or fragments thereof as defined in claims 2 and/or 15;

(c) a solid support as defined in claim 7;

adopted for carrying out the method of claims 1 to 8 and/or 15 to 19 optionally further comprising a standard and or suitable means for detection.

21. A kit comprising an inhibitor as defined in any one of claims 9 to 12 or a composition as defined in claim 13 or 14.

22. Use of the kit of claim 20 for carrying out the method of any one of claims 1 to 8 and/or 15 to 19.

23. Use of a peptide comprising a specific tau derived peptide as defined in SEQ ID Nos. 6, 7, 8 and/or 9 for carrying out the method of any one of claims 1 to 8 and/or

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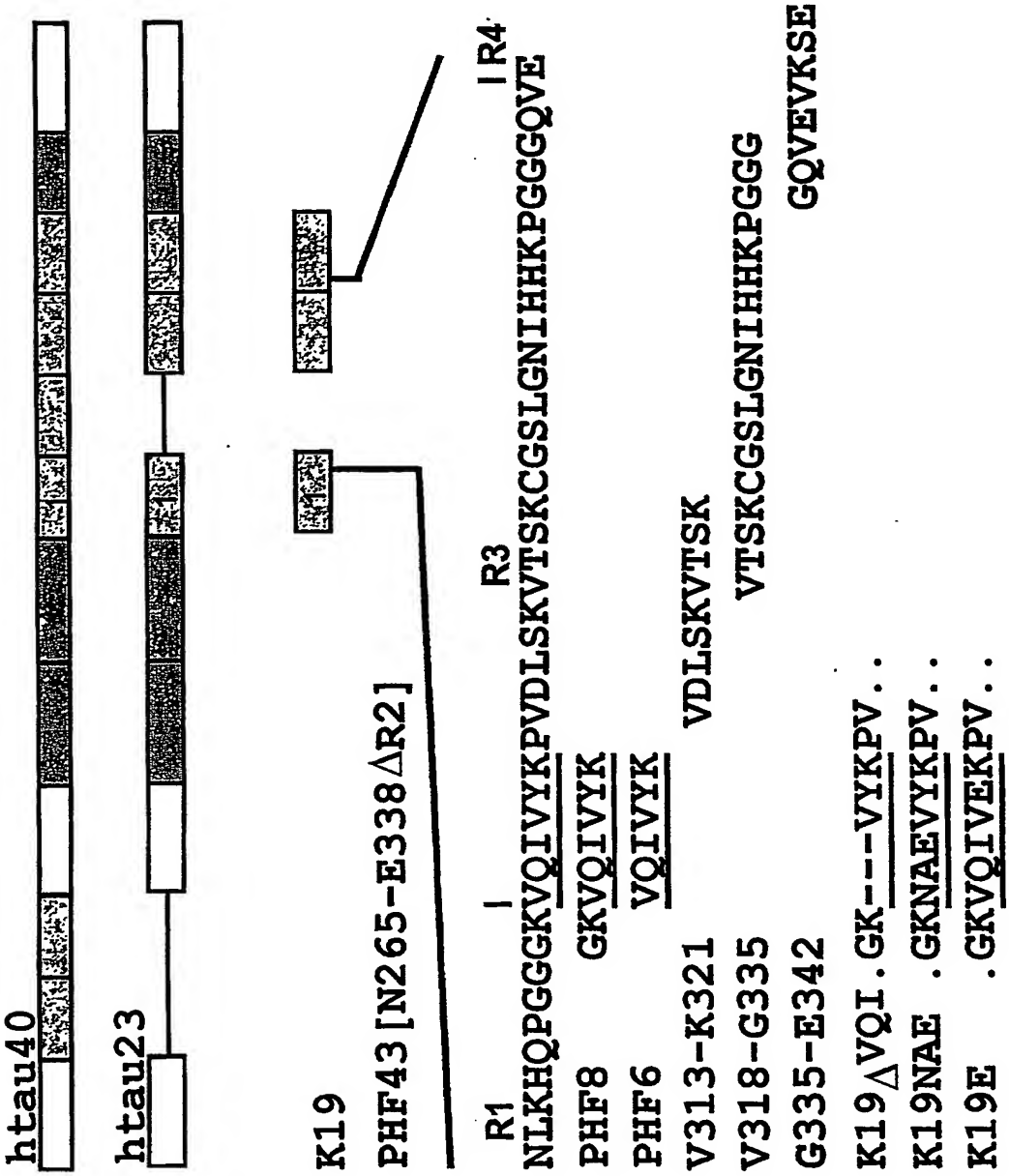
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Figure 1

K19 derived peptides and their ability for PHF assembly

Sample	Sequence	Aggr.
K19	Q244...PGGGKVQIVYKPV...N368	+
K19-Mut.	Q244...PGGGK---VYKPV...N368	-
	Q244...PGGGKNAEVYKPV...N368	-
	Q244...PGGGKVQIVEKPV...N368	-
K19/Chym.	QTAPVPMPDLKNVKSKIGSTENLKHQPGGGKVQIVY	-
	KPVDLSKVTSKCGSLGNIHHKPGGGQVEVKSEKLDF	-
	KDRVQSKIGSLDNITHVPGGGN	-
K19/GluC pH 4.0	QTAPVPMPDLKNVKSKIGSTE	-
	NLKHQPGGGKVQIVYKPVDLSKVTSKCGSLGNIHHKPGGGQVE	+
	VKSE	-
	KLDFKDRVQSKIGSLDNITHVPGGGN	-
K19/GluC pH 7.8	QTAPVPMPD	-
	LKNVKSKIGSTE	-
	NLKHQPGGGKVQIVYKPVD	*
	LSKVTSKCGSLGNIHHKPGGGQVE	-
	VKSEKLDFKDRVQSKIGSLDNITHVPGGGN	-
PHF8	GKVQIVYK	*
PHF6	VQIVYK	*
V_{313}-K_{321}	VDLSKVTSK	-
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Figure 3

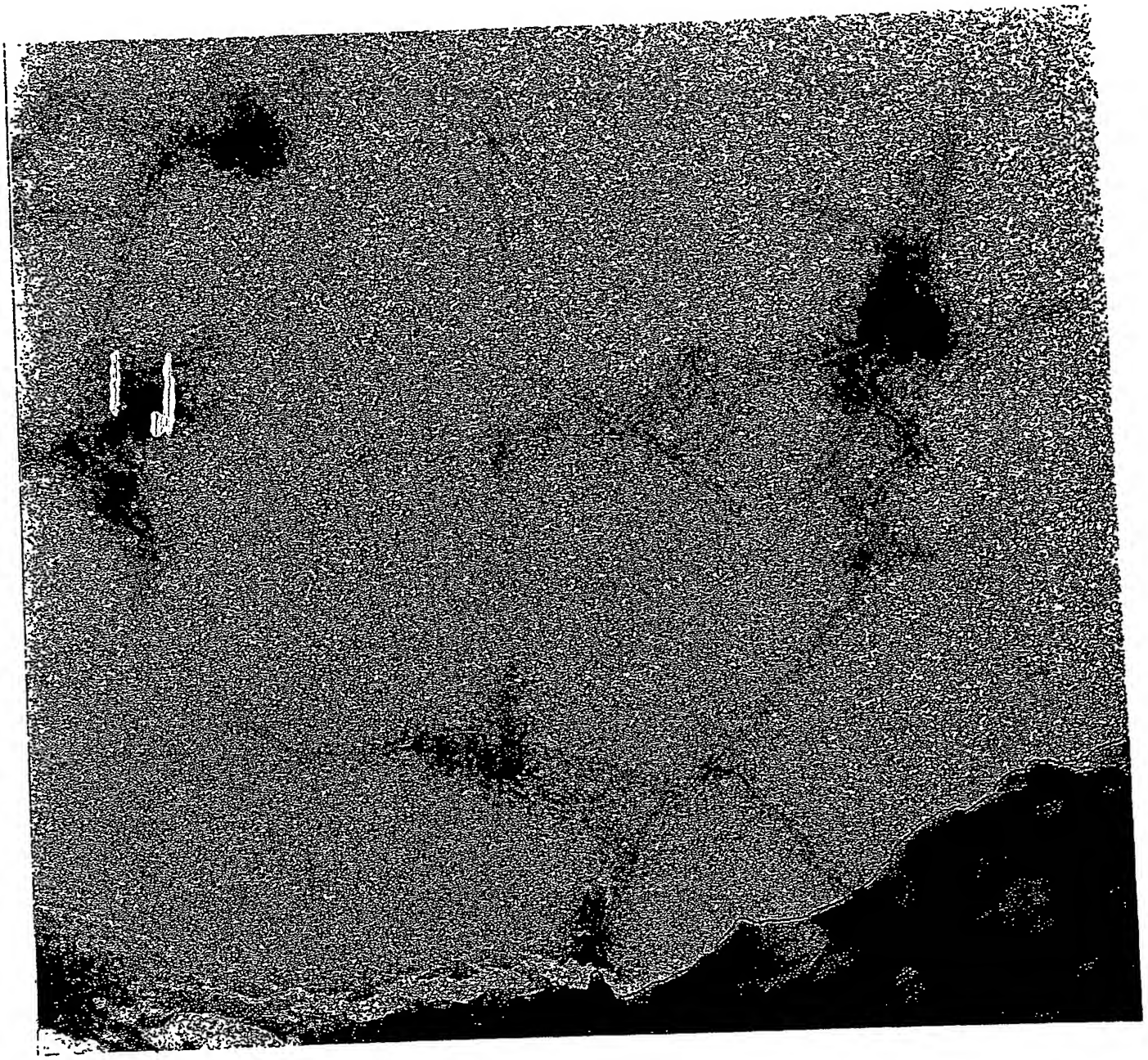


Figure 4b

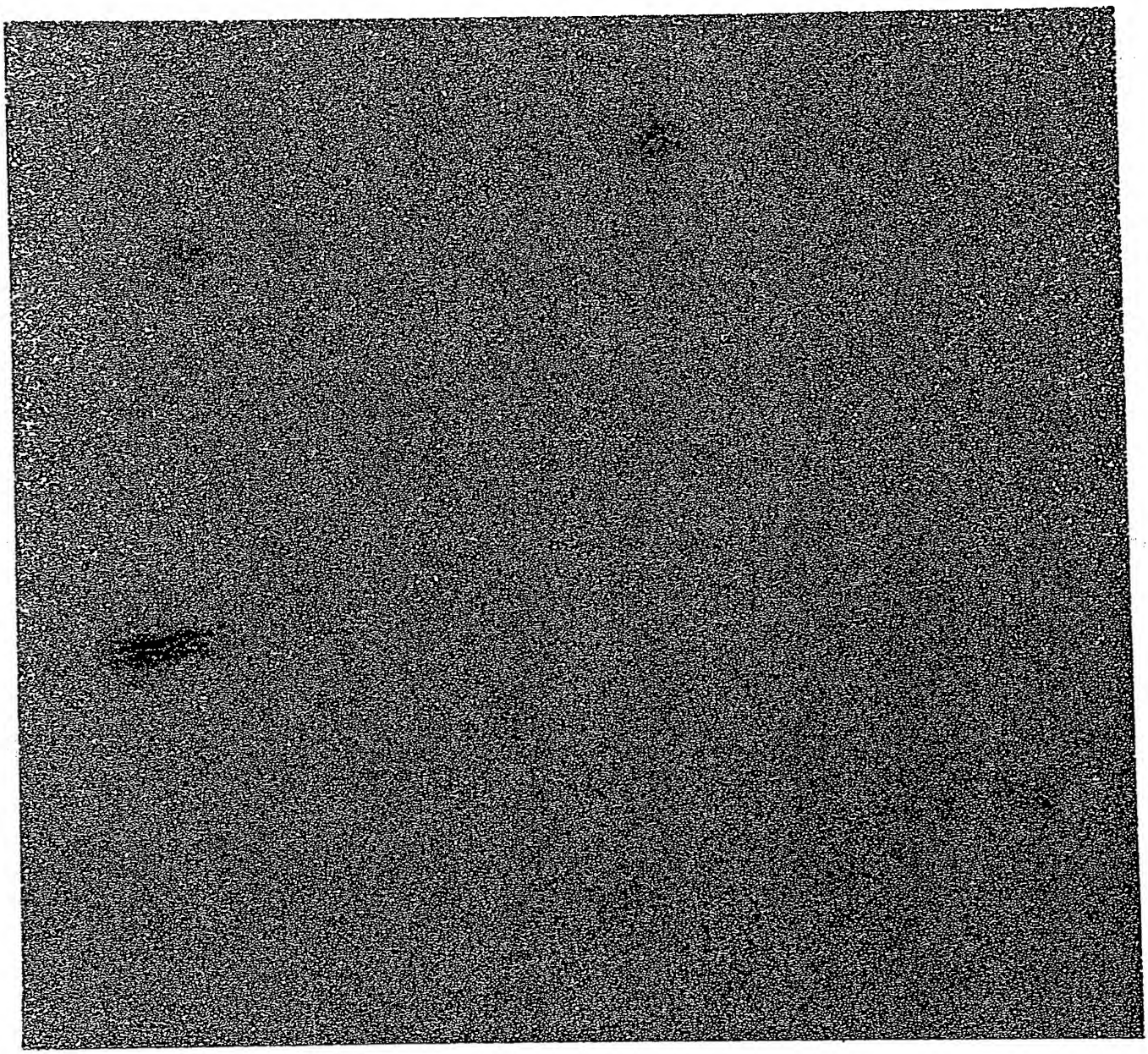


Figure 4d

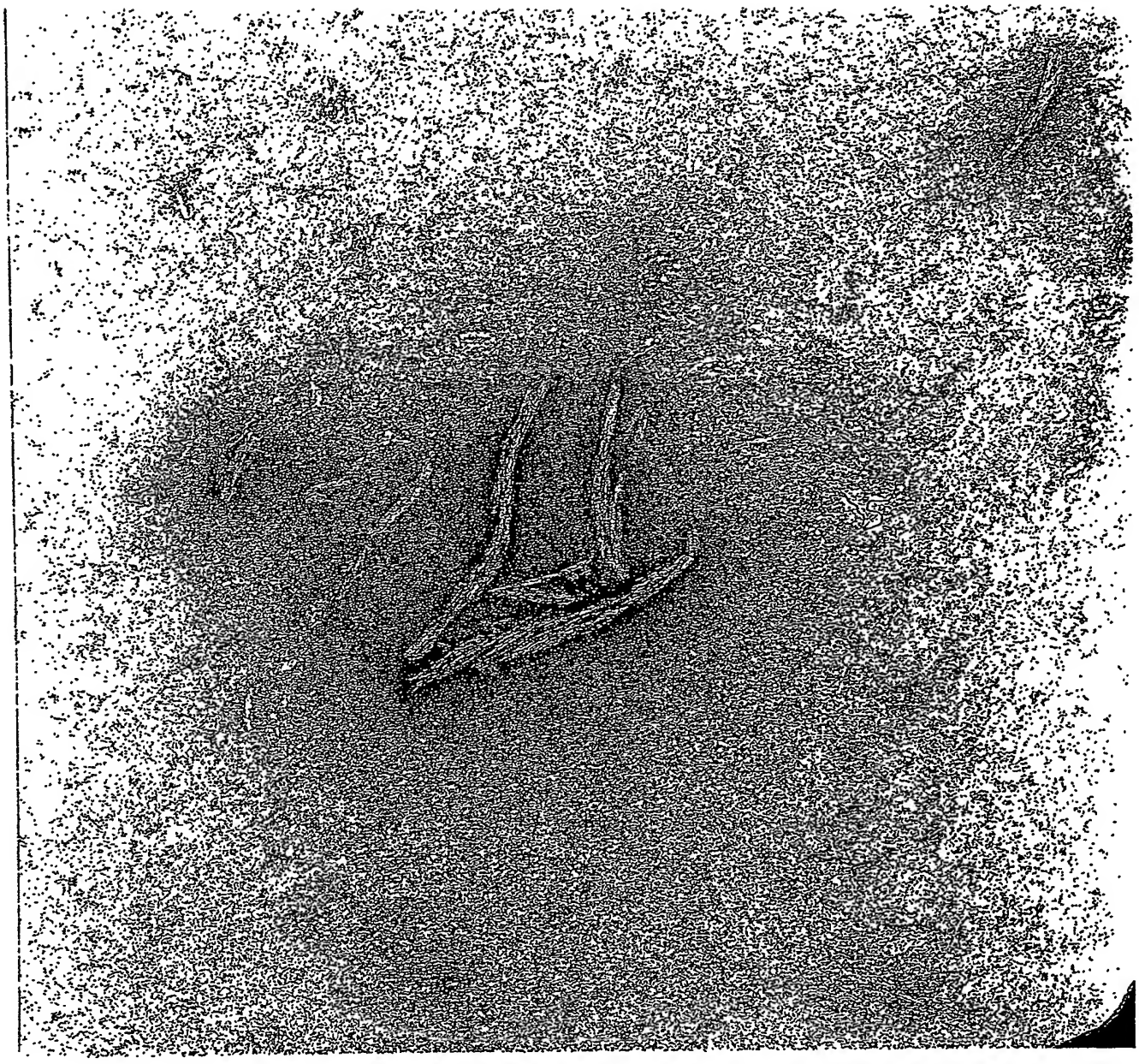


Figure 4f

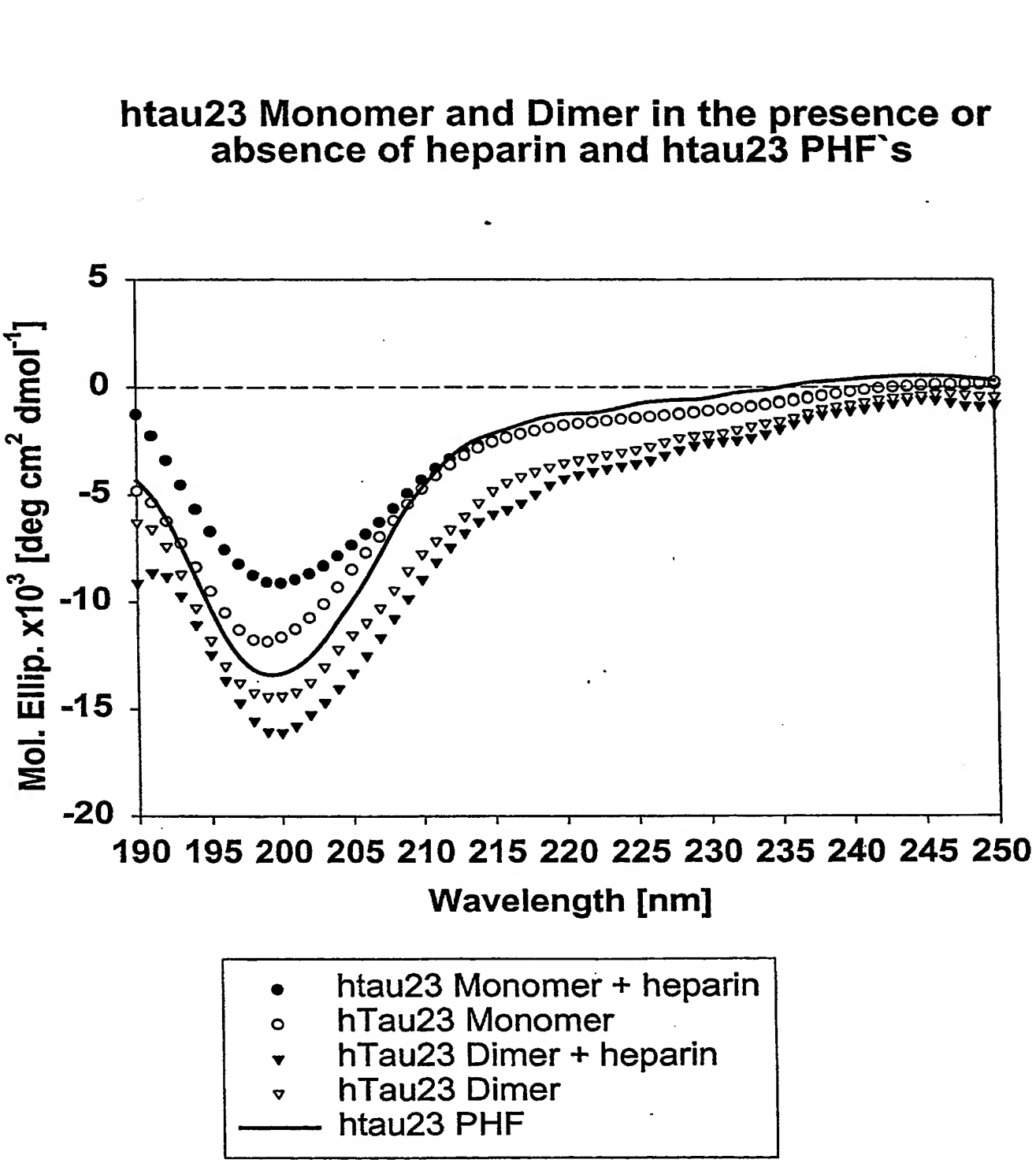

htau23 Monomer and Dimer in the presence or absence of heparin and htau23 PHF`s
Mol. Ellip. x10^3 [deg cm^2 dmol^{-1}]
5
0
-5
-10
-15
-20
190 195 200 205 210 215 220 225 230 235 240 245 250
Wavelength [nm]
htau23 Monomer + heparin
hTau23 Monomer
hTau23 Dimer + heparin
hTau23 Dimer
htau23 PHF

Figure 6a

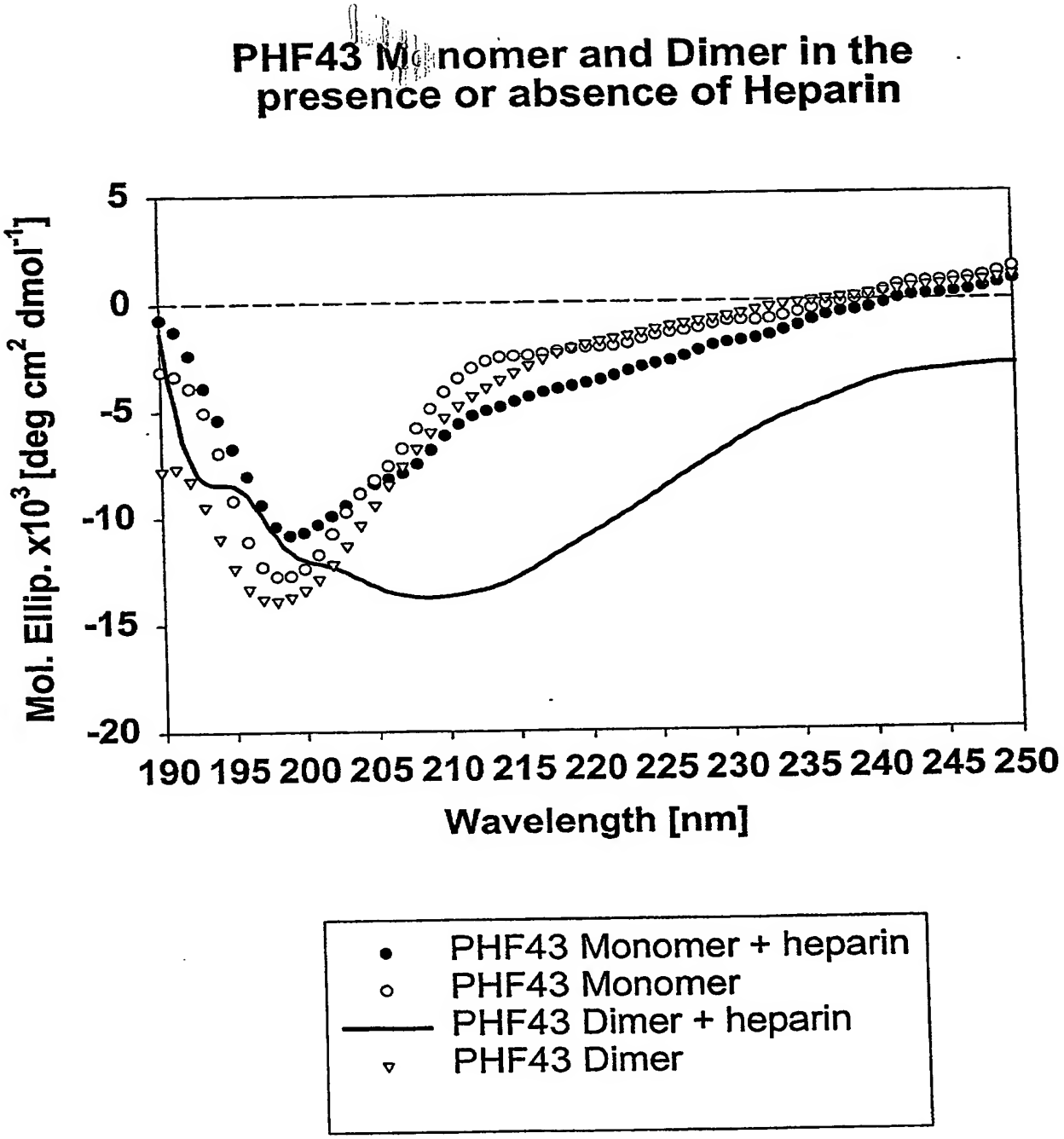
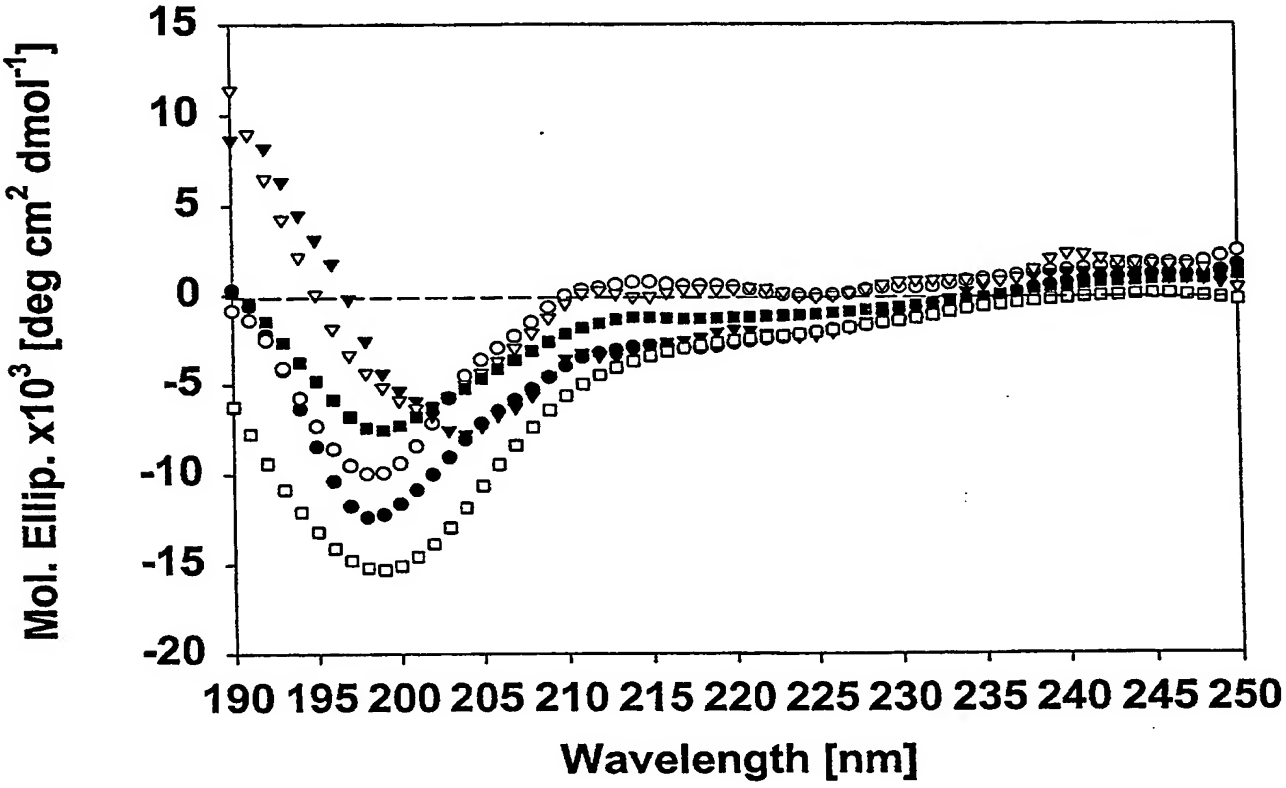

PHF43 Monomer and Dimer in the presence or absence of Heparin
Mol. Ellip. x10^3 [deg cm^2 dmol^-1]
5
0
-5
-10
-15
-20
190 195 200 205 210 215 220 225 230 235 240 245 250
Wavelength [nm]
PHF43 Monomer + heparin
PHF43 Monomer
PHF43 Dimer + heparin
PHF43 Dimer

Figure 6c

$V_{313}DLSKVTSK_{321}$, $V_{318}TSKCGSLGNIHHPGGG_{335}$, $G_{335}QVEVKSE_{342}$ in the presence and absence of heparin



- $V_{313}DLSKVTSK_{321}$ + heparin
- $V_{313}DLSKVTSK_{321}$
- $V_{318}TSKCGSLGNIHHKPGGG_{335}$ + heparin
- $V_{318}TSKCGSLGNIHHKPGGG_{335}$
- $G_{335}QVEVKSE_{342}$ + heparin
- $G_{335}QVEVKSE_{342}$

Figure 6e

Estimate of secondary structure content

Sample	Alpha helix	Beta sheet	Random coil
htau23 Monomer	13,6	18,3	68,1
htau23 Monomer + Heparin	14,3	20,0	65,7
htau23 Dimer	18,8	8,8	72,4
htau23 Dimer + Heparin	7,6	12,3	80,1
htau23 PHF	8,3	15,4	76,3
htau23 Dimer 50% TFE	62,4	6,4	31,2
htau23 PHF 50% TFE	65,2	10,3	24,5
K19 Monomer	12,1	21,5	66,4
K19 Monomer + hep.	28,1	1,7	70,2
K19 Dimer	13,0	13,6	73,4
K19 Dimer + hep.	2,3	22,4	75,3
K19 PHF	5,2	36,8	58,0
K19 Dimer 50 % TFE	67,2	32,8	0,0
K19 PHF 50 % TFE	34,0	32,2	33,8
PHF43 Monomer	6,6	24,7	68,7
PHF43 Monomer + hep.	9,0	30,4	60.6
PHF43 Dimer	4,3	23,2	72,5
PHF43 Dimer + hep.(PHF)	0,0	55,2	44,8
PHF43 Dimer 50% TFE	38,5	27,3	34,2
PHF43 PHF 50% TFE	9,4	48,3	42,3
GKVQIVYK	8,2	23,7	68,1
GKVQIVYK + hep.	5,4	33,1	61,5
VQIVYK	17,1	5,7	77,2
VQIVYK +hep.	6,6	58.1	35.3
VDLSKVTSK	5,2	22,7	72,1
VDLSKVTSK + hep.	0,0	29.9	70,1
VTSKCGSLGNIHHKPGGG	15,2	22,7	62,1
VTSKCGSLGNIHHKPGGG + hep.	13,5	29.9	56,6
GQVEVKSE	0,0	25,3	74,7
GQVEVKSE + hep.	8,8	22,7	68,5

Figure 7

PHF43 Excitation-Spectrum

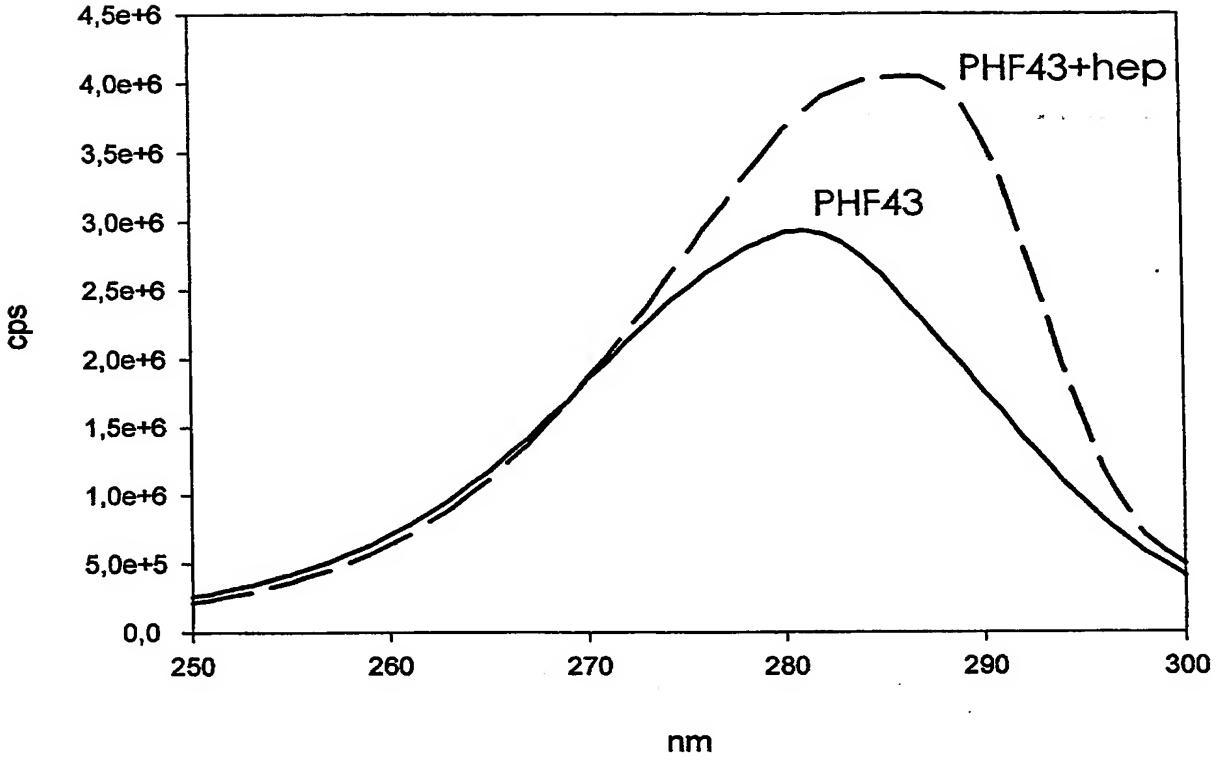


Figure 8b

PHF6 Excitation-Spectrum

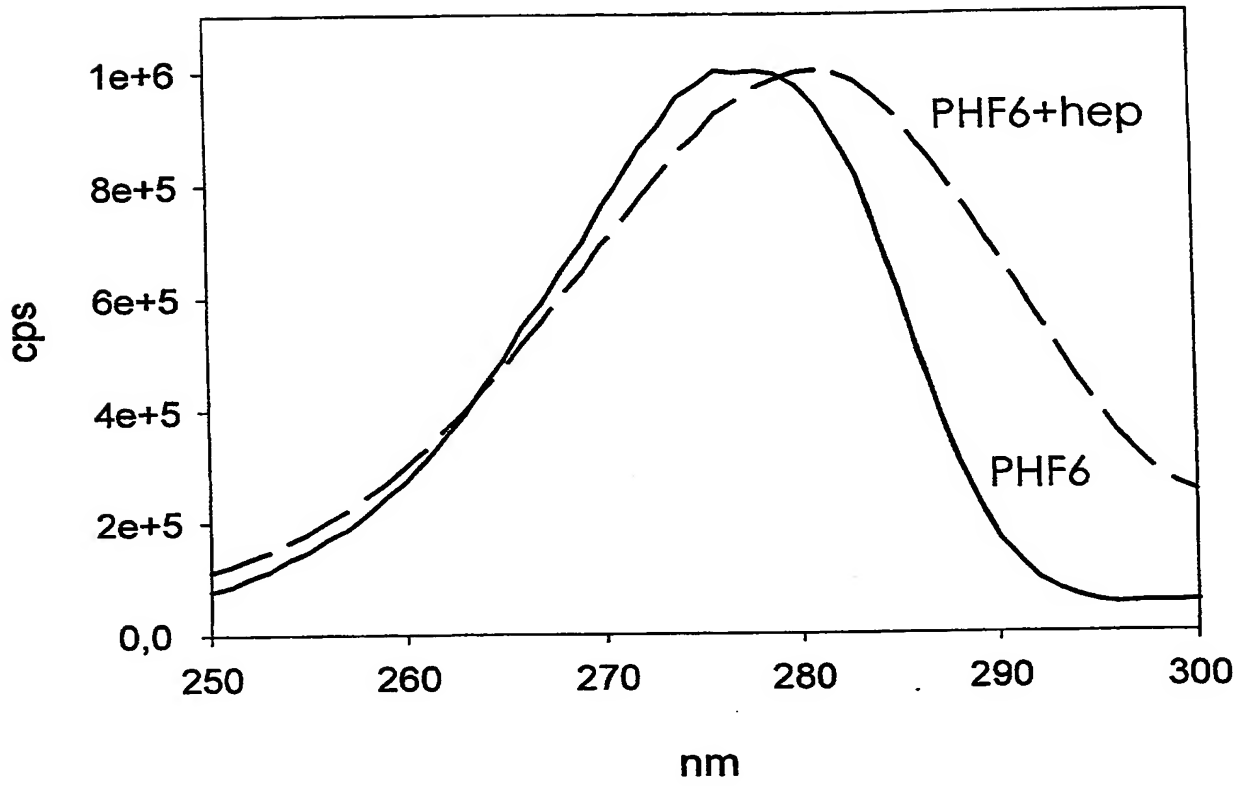


Figure 8d

Abstract

The pres nt invention relates to a method for identifying and obtaining an inhibitor, capable of modifying the PHF ("paired helical filaments") formation comprising the steps of (a) incubating a peptide comprising a specific tau derived peptide as defined herein or a fragment(s) thereof with a compound to be screened under conditions which permit assembly of said tau-derived peptides into nucleation sites for PHF assembly and/or into aggregation products; and (b) detecting the presence, decrease, or absence of nucleation sites for PHF assembly and/or the presence, decrease or absence of said aggregation products wherein said absence and/or decrease is indicative for putative inhibitors for PHF formation. Furthermore, the present invention provides inhibitors identified or obtained by said method as well as compositions comprising said inhibitor, wherein said composition is preferably a diagnostic and/or a pharmaceutical composition. The present invention further relates to a method for detecting and/or measuring PHF formation comprising the steps of (a) incubating a peptide comprising a specific tau derived peptide as defined herein or (a) fragment(s) thereof, with tau-proteins and/or fragments thereof under conditions which permit assembly of tau-proteins and/or fragments thereof into PHFs; and (b) detecting the presence, absence, decrease or increase of PHFs and/or nucleation sites of PHF assembly. Additionally, the present invention provides for kits and uses for carrying out the method of the present invention.